PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Decreased Prevalence of Diabetes in Marijuana Users: Cross-sectional Data from the National Health and Nutrition Examination Survey (NHANES) III
AUTHORS	Tripathi B. Rajavashisth, Magda Shaheen, Keith C. Norris, Deyu Pan, Satyesh K. Sinha, Juan Ortega and Theodore C. Friedman

VERSION 1 - REVIEW

REVIEWER	Dr M Z Chen MRCP Clinical Research Fellow Diabetes and Metabolism University of Bristol United Kingdom I have no competing interests.
REVIEW RETURNED	20/11/2011

GENERAL COMMENTS	Interesting and provocative findings with very clear key message. No concerns.

REVIEWER	Paul Lee MBBS FRACP PhD Endocrinologist and Research Fellow School of Medicine University of Queensland Australia
REVIEW RETURNED	05/12/2011

THE STUDY	I have some questions regarding exclusion criteria which I have discussed in my comments to the authors.
GENERAL COMMENTS	Rajavashisth et al evaluated the association between marijuana use and diabetes mellitus (DM) in a cross-sectional analysis of demographic, anthropometric and biochemical data obtained from 10896 adults. Prevalence for diabetes mellitus was lower among marijuana users after adjusting for potential confounders. The authors conclude marijuana use was independently associated with a lower prevalence of DM. Obesity and DM are reaching epidemic proportion worldwide. Pathogenesis is complex and involves multiple organs in a dysregulated hormonal and inflammatory milieu. There has been substantial increase in research interest in recent years to understand central pathways that mediate both hormonal and inflammatory changes. The current study is therefore timely as it explores the potential metabolic benefits of cannabinoid system agonism. The authors are meticulous in their analysis to address

	known limits and weaknesses of cross-sectional design. Most metabolic and demographic confounders have been considered and marijuana use persists as a significant associating factor, despite scrutiny of data by several analytical methods. Within the confines of a cross-sectional design, authors have provided tantalizing evidence supporting a protective role of marijuana use in the development of DM, and at the same time emphasized causality cannot be proven until prospective data become available in future. I have the following suggestions for the authors to consider: INTRODUCTION 1) The metabolic benefits of CB1 antagonism (eg by rimonabant), contrasts with the hypothetical benefits of marijuana-mediated CB1 agonism, and it may be worth mentioning in this section. METHODS 1) 1333 subjects between 20-59 were excluded, totaling 12% of the final sample. 959 answered “not applicable” to the question “Have you ever used marijuana”, 373 left it blank while 1 answered “Don’t know”. Given the potential social stigma and dysfunctional lifestyle sometimes associated with recreational drug use, is it possible that the excluded subjects represent the heaviest marijuana users who did not respond to the question and may potentially have more untreated/undiagnosed metabolic conditions/dysglycaemia? Apparently those with missing laboratory data were also excluded but it was not clear from Supplement Fig 1 where this group fell under in the flow diagram. 2) In the assessment of DM status, how was gestational diabetes (GDM) accounted for? One might expect a lower prevalence of marijuana use among pregnant women, therefore contributing to the observed “higher DM prevalence” among “marijuana non-users”. This is congruent with the finding of the trend for patients with history of DM by self-report who were euglycemic at the time of sampling to be associated with a lower rate of non-marijuana use. 3) Multivariate logistic regression was used to adjust for confounding variables and odds ratio was reported. However marijuana users and non-users share common characteristics. The authors may consider using propensity scores to account for unbalanced variables when comparing the two groups. This allows the matching of each marijuana user with a non-user with a similar propensity score. Such propensity score–based methods may help to adjust for selection bias caused by confounding variables (eg alcohol) associated with both marijuana exposure and outcome. RESULTS 1) In the evaluation of DM status, authors examined whether DM as diagnosed by self-report as compared to laboratory evidence of hyperglycaemia was correlated with different prevalence of marijuana use. It would be informative to present the proportion of non-, past and current marijuana users among subjects classified to have DM based on self-report and/or laboratory testing. 2) A strong association was seen between marijuana use and lower DM prevalence/lower fasting glucose levels. However no association was seen between the use of marijuana and other chronic metabolic disorders such as hypertension, which shared similar risk factors to

	DM. What is the relationship between glucose levels with BMI, lipids and inflammatory markers, and are these factors associated with a higher prevalence of hypertension, strokes and myocardial infarction? While the lack of association between marijuana use and chronic metabolic/vascular disorders could be due to a type II error, it would be reassuring to see known negative correlations between these classic risk factors and metabolic/vascular disorders demonstrated, to support selection/analysis validity. DISCUSSION 1) As authors have indicated, prospective studies in humans are needed to determine a causal relationship between cannabinoid receptor activation and DM. A recent randomized placebo-controlled double-blind clinical trial (Selvarajah et al. Diabetes Care 2010; 33: 128-30) evaluated the effects of cannabis-based medicinal product in diabetic neuropathy over a 6 months period. Changes in HbA1c were not reported. While the sample size was small (N=38), investigators of the study might have collected data on glycaemic changes following treatment. 2) A recent study by Kerr et al (J Stud Alcohol Drugs 2010; 71:515-25) may be worth discussing. It showed that low quantity alcohol intake could be protective against DM, after controlling for confounders, including marijuana use. How do authors interpret these results in light of findings presented in current manuscript?

VERSION 1 – AUTHOR RESPONSE

Reviewer: Dr M Z Chen MRCP
Clinical Research Fellow
Diabetes and Metabolism
University of Bristol
United Kingdom

I have no competing interests.

Interesting and provocative findings with very clear key message.

No concerns.

We thank Dr. Chen for his/her positive comments.

Reviewer: Paul Lee MBBS FRACP PhD
Endocrinologist and Research Fellow
School of Medicine
University of Queensland
Australia

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prevalence of DM.

Obesity and DM are reaching epidemic proportion worldwide. Pathogenesis is complex and involves multiple organs in a dysregulated hormonal and inflammatory milieu. There has been substantial increase in research interest in recent years to understand central pathways that mediate both hormonal and inflammatory changes. The current study is therefore timely as it explores the potential metabolic benefits of cannabinoid system agonism. The authors are meticulous in their analysis to address known limits and weaknesses of cross-sectional design. Most metabolic and demographic confounders have been considered and marijuana use persists as a significant associating factor, despite scrutiny of data by several analytical methods. Within the confines of a cross-sectional design, authors have provided tantalizing evidence supporting a protective role of marijuana use in the development of DM, and at the same time emphasized causality cannot be proven until prospective data become available in future.

We thank Dr. Lee for his positive comments.

I have the following suggestions for the authors to consider:

INTRODUCTION

The metabolic benefits of CB1 antagonism (eg by rimonabant), contrasts with the hypothetical benefits of marijuana-mediated CB1 agonism, and it may be worth mentioning in this section.

We discussed the differences between CB1 antagonism (rimonabant) and marijuana-mediated CB1 agonism in he discussion on p. 15.

METHODS

1) 1333 subjects between 20-59 were excluded, totaling 12% of the final sample. 959 answered “not applicable” to the question “Have you ever used marijuana”, 373 left it blank while 1 answered “Don’t know”. Given the potential social stigma and dysfunctional lifestyle sometimes associated with recreational drug use, is it possible that the excluded subjects represent the heaviest marijuana users who did not respond to the question and may potentially have more untreated/undiagnosed metabolic conditions/dysglycaemia? Apparently those with missing laboratory data were also excluded but it was not clear from Supplement Fig 1 where this group fell under in the flow diagram.

We excluded those with missing laboratory data from the analyses list wise. We added a note to the diagram.

2) In the assessment of DM status, how was gestational diabetes (GDM) accounted for? One might expect a lower prevalence of marijuana use among pregnant women, therefore contributing to the observed “higher DM prevalence” among “marijuana non-users”. This is congruent with the finding of the trend for patients with history of DM by self-report who were euglycemic at the time of sampling to be associated with a lower rate of non-marijuana use.

The study included 151 pregnant women (1.5%). Of the 151 pregnant women, 8 women had diabetes. There was no difference in the use of marijuana by diabetes status. Because of the low number in the diabetic category, we included them in the analysis.

We ran the analysis with and without the pregnant women and we found no statistical difference in the results (the odds ratio for the final model was 0.32, 0.38, and 0.82 for the total sample, age group 41-59 years, and age group 20-40 years respectively excluding the pregnant women. 0.360.37and

0.93including the pregnant women and thus reached the same conclusion.

3) Multivariate logistic regression was used to adjust for confounding variables and odds ratio was reported. However marijuana users and non-users share common characteristics. The authors may consider using propensity scores to account for unbalanced variables when comparing the two groups. This allows the matching of each marijuana user with a non-user with a similar propensity score. Such propensity score–based methods may help to adjust for selection bias caused by confounding variables (eg alcohol) associated with both marijuana exposure and outcome.

We analyzed the data using the propensity score matching (nearest neighbor and kernel matching) in STATA as suggested to help adjust for selection bias caused by confounding variables. We found very similar results that showed a lower prevalence of diabetes mellitus among marijuana-users relative to non-users. The average treatment effect for the treated for the total sample = -0.024, bootstrap standard error= 0.005, and t-statistics=-4.46, $p<0.05$ (i.e., marijuana users had significantly lower prevalence of diabetes mellitus).

We added the propensity score to the logistic regression model and found that marijuana users had significantly lower odds of diabetes mellitus than non-users (OR=0.54, 95% confidence level=0.40-0.73, p=0.001).

In another analysis we added the propensity score to the model as inverse weight and found the OR=0.52 (95% confidence level=0.39-0.71, p=0.001).

We also added the propensity score blocks (N=8) that satisfy the balancing property to the logistic regression model and found the OR=0.53 (95% confidence level=0.40-0.73, p=0.001).

We also added the propensity score as quartiles to the logistic regression model and found that OR=0.51, 95% confidence level=0.38-0.69, p=0.001.

We also performed stratified analysis by age group. For age group 41-59, adding the propensity score as quartiles to the model, we found the OR=0.55 (95% confidence level=0.35-0.88, p=0.012).
For age group 41-59, adding the propensity score as quartiles to the model, we found the OR=0.88 (95% confidence level=0.53-1.47, $p>0.05$).

RESULTS
1) In the evaluation of DM status, authors examined whether DM as diagnosed by self-report as compared to laboratory evidence of hyperglycaemia was correlated with different prevalence of marijuana use. It would be informative to present the proportion of non-, past and current marijuana users among subjects classified to have DM based on self-report and/or laboratory testing.

We have added the percentage of non-, past- and current-marijuana users (four groups) among subjects classified to have DM based on self-report and/or laboratory testing to Supplemen Table 3.

2) A strong association was seen between marijuana use and lower DM prevalence/lower fasting glucose levels. However no association was seen between the use of marijuana and other chronic metabolic disorders such as hypertension, which shared similar risk factors to DM.
What is the relationship between glucose levels with BMI, lipids and inflammatory markers, and are these factors associated with a higher prevalence of hypertension, strokes and myocardial infarction? While the lack of association between marijuana use and chronic metabolic/vascular disorders could be due to a type II error, it would be reassuring to see known negative correlations between these classic risk factors and metabolic/vascular disorders demonstrated, to support selection/analysis validity.

There was a statistically significant relationship between glucose level and BMI, lipids, and inflammatory markers where high level of glucose was associated with high levels of BMI, total cholesterol, triglyceride, and C-reactive protein and low level of LDL ($p<0.05$). These are all consistent with known associations with diabetes. Additionally, these factors were all associated with a high prevalence of hypertension. For stroke, only levels of total cholesterol, triglyceride, and C-reactive protein were associated with high prevalence of stroke.
For myocardial infarction, only levels of BMI and triglyceride were associated with high prevalence of myocardial infarction. (Please see table below).

Prevalence of diseases by risk factors

	%FBG>126	%Hypertension	%Stroke	%Myocardialinfarction
Overall	3.0	22.0	0.7	1.3
BMI (kg/m2)				
<30	2.0*	16.0*	0.6	1.0
≥30	8.0	44.0	1.0*	2.4
HDL (mg/dL)				
>40	2.0*	19.0*	0.5	1.0
≤40	5.0	27.0	1.0	2.0
Total Cholesterol (mg/dL)				
<240	3.0*	20.0*	0.5*	1.0
≥240	6.0	35.0	2.0	2.0
Triglyceride (mg/dL)				
<200	2.0*	19.0*	0.5*	1.0*
≥200	9.0	37.0	2.0	3.0
CRP (mg/dL)				
<0.5	2.0*	19.0*	0.5*	1.0
≥0.5	9.0	37.0	2.0	2.0

* $p<0.05$

As these associations are not related to our paper that is characterizing the associations between marijuana and diabetes, we did not include them in our paper. They are, however, consistent with known associations for these diseases and validate our model.

DISCUSSION

1) As authors have indicated, prospective studies in humans are needed to determine a causal relationship between cannabinoid receptor activation and DM. A recent randomized placebo-controlled double-blind clinical trial (Selvarajah et al. Diabetes Care 2010; 33: 128-30) evaluated the effects of cannabis-based medicinal product in diabetic neuropathy over a 6 months period. Changes in HbA1c were not reported. While the sample size was small (N=38), investigators of the study might have collected data on glycaemic changes following treatment.

We thank the Dr. Lee for making us aware of this article and have cited it on p. 15 of the revised manuscript.

2) A recent study by Kerr et al (J Stud Alcohol Drugs 2010; 71:515-25) may be worth discussing. It showed that low quantity alcohol intake could be protective against DM, after controlling for confounders, including marijuana use. How do authors interpret these results in light of findings presented in current manuscript?

In an earlier version of this manuscript, we devoted several paragraphs to the interaction between alcohol and marijuana and their correlations with diabetes. However, to comply with space limitations, we removed that section. We have now, however, added the paper by Kerr et al. showing that light alcohol use protects against diabetes on p.13 of the revised manuscript.

We hope our revised manuscript has adequately addressed the recommendations and is found suitable for publication in BMJ Open. We are submitting a revised electronic submission of our manuscript in PDF format.

We look forward to hearing from you after the re-review process.

Sincerely yours,

Magda Shaheen

Associate Professor
Charles Drew University

VERSION 2 – REVIEW

REVIEWER	Paul Lee MBBS FRACP PhD Endocrinologist and Research Fellow School of Medicine University of Queensland Australia
REVIEW RETURNED	20/01/2012

GENERAL COMMENTS	The authors have addressed my concerns to my satisfaction. This is a novel, interesting and hypothesis generating study with clinically relevant implications. This manuscript is likely to stimulate intervention studies and tests of cannabinoid receptor activation to improve glycaemia.